Behavioral and hormonal changes following social instability in young rhesus macaques *(Macaca mulatta)*

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Running title: Social instability in young macaques

Abstract

Social instability (SI) occurs when there is competition over social status. Reduced certainty of social status can lead to heightened aggression, which can increase physiological stress responses, as individuals prepare to fight for their social status. However, adults can take proactive coping mechanisms to reduce the physiological stress induced by SI, such as increasing affiliation. Very little is known, however, about the behavioral and hormonal effects of SI early in development. Filling these gaps in knowledge would add to the fields of primatology and developmental and comparative psychology. We conducted an opportunistic study of a peer group of 18 rhesus macaque (*Macaca mulatta*) yearlings before and during SI. We used social network analysis to measure individuals' dominance certainty (DC, in their aggressive and submissive network) and their position in affiliative networks (grooming and play) and analyzed hair cortisol concentrations (HCCs). As predicted, during SI, we observed a decrease in DC, indicating that individuals had less stable dominance positions. As well, during SI, we observed increased rates of social grooming and decreased rates of social play, reflecting potential coping mechanisms. More socially connected individuals in social grooming and social play networks received higher levels of coalitionary support. Contrary to predictions, DC did not predict HCCs; rather individuals that were more connected in the social play network exhibited smaller increases in HCCs during SI, revealing a potential buffering effect of social play. Our results underscore the need for further research on the effects of SI during ontogeny.

Keywords: instability, social network, hair cortisol, eigenvector centrality, play

Introduction

Across a wide range of animal species, including humans, dominance hierarchies structure the organization of social groups (Grosenick et al., 2007; Hawley & Little, 1999; Williamson et al., 2016). When a new social group is formed, individuals may use a variety of strategies to establish dominance ranks, including sensory cues (e.g., auditory, olfactory, or visual), in addition to aggression. When utilizing aggression, "a pecking order" can often be established relatively quickly, even within minutes (Issa et al., 1999; Meese & Ewbank, 1973; Wooddell et al., 2017). The presence of a social hierarchy is beneficial to prevent prolonged and intense fighting, as the establishment of a social relationship can govern future interactions, including approach and retreat behaviors (Casey et al., 2015).

Although dominance relationships can remain stable for an extended amount of time, they are not completely static. They may be maintained via bouts of fighting or periods of conflict that can result in temporary social instability (SI), during which dominance ranks may be fluctuating and unstable. Accordingly, SI is commonly assessed by measuring changes in dominance ranks or the proportion of bidirectional interactions (e.g., Neumann et al., 2011). In free-ranging animal populations, SI commonly occurs when immigrant males attempt to enter into a new group and challenge the resident males, which may lead to excessive violence, including infanticide (Beehner & Berman, 2008; Hrdy, 1974; Pusey & Packer, 1994). Thus, in captivity, paradigms to induce SI (in order to study its physiological effects) are introductions of novel males or revolving group memberships (e.g., Almeida et al., 2014; Capitanio & Cole, 2015; Guibert et al., 2010; Manuck et al., 1983) and group formations (e.g., Dise & Goldina, 2017; Linden et al., 2019). However, SI is not just limited to new individuals entering groups, as rank changes can occur even in established social groups (both captive and free-ranging) with

long-term dominance relationships (e.g., Ehardt &Bernstein, 1986; Beisner et al., 2015; Higham & Maestripieri, 2010; Kaburu et al., 2013; Meese & Ewbank, 1972; Perry, 1998; Setchell & Dixson, 2001).

During SI, individuals are at risk for severe traumas, infanticide and other reproductive failures, and even death (e.g., Dettmer et al., 2015), and decades of research have examined the effects that SI has on animals' physiological regulation, with a particular focus on the link between SI and glucocorticoid (GC) levels (Bartoš et al., 2010; Haller et al., 1999; Van Meter et al., 2009). GCs (i.e., cortisol and corticosterone) are steroid hormones that help animals mobilize energy reserves whenever their body needs it, such as during a stress response. Since GCs are part of the "fight or flight" response, they are expected to be elevated during periods of potentially intense fighting seen with contested dominance ranks because these metabolic demands require immediate energy expenditure (Muller & Wrangham, 2004). Accordingly, several studies have found that GC levels (measured by blood, urine, fecal, or hair) are elevated during periods of SI (Engh et al., 2006; Haller et al., 1999; Higham et al., 2013; Mendonça-Furtado, et al., 2014; Sapolsky, 2005; Vandeleest et al., 2019; Wooddell et al., 2016; but see Milich et al., 2018). The heightened GCs are particularly salient for vulnerable individuals, for example, lactating mothers at risk of losing their infants to infanticide during male takeovers (Crockford et al., 2008; Engh et al., 2006; Wittig et al., 2008) and high-ranking individuals at risk of losing their social standing (Higham et al., 2013; Sapolsky 2005; Wooddell et al., 2016). However, given GCs' role in suppressing the immune system and inflammatory response, chronic high levels of GCs can result in immune suppression, infertility, and cardiovascular impairments (Sapolsky, 2005), and individuals can take proactive coping strategies to limit this GC surge, such as narrowing their social networks to focus on a few preferred social partners

(Crockford et al., 2008; Engh et al., 2006; Wittig et al., 2008) or enhancing grooming rates in general (Wooddell et al., 2016). In these studies of adult primates, social affiliation seemed to buffer individuals during periods of social unrest.

Importantly for group-living primates, adults are not the only individuals witnessing or experiencing SI. Many wild and captive troops contain multi-generational members, including immatures (i.e., infants and juveniles). However, it still remains unclear how SI affects both behavioral and hormonal profiles in juveniles and whether coping mechanisms against chronic stress emerge early in life, which would be an adaptive strategy for preparing for stressful events in adulthood.

To examine how SI influences both behavioral and hormonal coping mechanisms, we conducted an opportunistic study of a peer group of rhesus macaques (all approximately 1-year old) after social group formation and relocation. We have previously studied this group extensively to examine the predictors of rank acquisition (Wooddell et al., 2017) and the development of social networks (Wooddell et al., 2019). However, until now we did not have the capability to study their hormonal regulation and potential coping mechanisms during SI, which was the aim of the present study. This period of SI provides an excellent opportunity to examine how young primates adapt to SI both behaviorally and hormonally, especially in the absence of kin support. Given that mothers and kin can be a significant social buffer for developing primates (Hennessy et al., 2009; Kikusui et al., 2006; Sanchez et al., 2015), studying a peer group allows us to examine individual-level coping mechanisms, which could provide a relevant monkey model for the study of human development in the absence of kin support (e.g., foster children, orphans, institutionalized children) or in settings where peer interactions predominate (e.g., school settings: Kim et al., 2006; 2009; Tschann et al., 1996). We used dominance interactions

to calculate dominance hierarchies and certainty (DC) and relied on social grooming and social play data to construct social networks. For the present study, we also analyzed hair samples to measure hair cortisol concentrations (HCCs), a measure of chronic GC production (Davenport et al., 2006; Meyer & Novak, 2012) that reflects aggregate HPA axis activity over the preceding months. We predicted that 1) DC would decrease during SI, reflecting the presence of or an increase in unstable dominance relationships. However, if young monkeys can utilize affiliative social relationships to modify their DC, we then predicted that 2a) individuals more central in affiliative social networks (grooming and play) would have greater DC, as 2b) individuals with more social connections may benefit from a larger network of coalitionary support. Given that weaker DC is associated with unstable dominance relationships, which may equate to more intense fighting, we also predicted that 3) individuals with less DC should have higher HCCs, particularly during SI. However, if immatures can use affiliation as a coping strategy, we would predict that 4) lower HCCs during SI should be associated with a higher centrality in social grooming and social play networks.

Methods

Subjects and housing

Subjects were 14 male and 4 female rhesus macaques (*Macaca mulatta*, N=18) born between March and May 2015 at the Laboratory of Comparative Ethology at the NIH Animal Care Center in Poolesville, Maryland. Monkeys were reared at this facility through August 2016, when they were approximately 1.5 years old (see Wooddell et al., 2017; 2019, for detailed methodology). All procedures described adhered to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the National Institute of Child Health and Human Development Animal Care and Use Committee.

Nine of the subjects (eight males and one female) were reared in a 2.0 ha naturalistic outdoor, open-air enclosure (field station), which has previously been described in detail (e.g., see Dettmer et al., 2014; Wooddell et al., 2016; 2017). The troop consisted of approximately 80 mixed-sex semi-free ranging rhesus macaques (age range: 0-18 years) from three different matrilineal kinship lines (Wooddell et al., 2016; 2017). Offspring acquired their mothers' dominance ranks in infancy, as dominance ranks among peers were settled even before 8 months of age (Wooddell et al., 2016; 2017; 2019). Families contained infants/siblings, mothers, aunts, grandmothers, and even great-grandmothers, reflecting the naturalistic multigenerational (MG) social structure. Following our previous approach (Wooddell et al, 2017; 2019), these subjects were referred to as MG subjects, reflecting this type of early social experience.

The other nine subjects (six males, three females) were born and reared into one of three social groups, each of which consisted of 10-12 same aged females, one adult male, and several same-aged infants (detailed in Wooddell et al., 2017; 2019). Infants born into these groups were paternal half-siblings. Contrary to the MG subjects, these groups exhibited very little age diversity, with only maternal and infant generations, and were thus classified as unigenerational (UG) subjects (Wooddell et al., 2017; 2019). It is important to note, however, that MG and UG subjects not only differed in the complexity of rearing in terms of generations, but also in group size: MG subjects were reared in a much larger group size than UG subjects.

Peer group formation and social instability (SI)

In January 2016, the nine MG immatures and their mothers were removed from the field station due to a rare overthrow of the social hierarchy in the field station (Wooddell et al., 2017). These nine subjects (all approximately 8-months old) were the infants of females who suffered severe aggression and trauma but did not receive trauma themselves. They were all from the

same matriline. The other nine subjects, UG immatures, were removed from their mothers at approximately 8-months of age to adhere to standardized laboratory procedures to place them into a group with same-aged peers (see Dettmer et al., 2012).

All 18 subjects (nine MG and nine UG) were placed together in a new social group, as previously described in detail (Wooddell et al., 2017; 2019), and then relocated in May 2016 (when all subjects were approximately 1-yr of age), due to management decisions unrelated to the research objectives. The relocation resulted in a turnover of the peer group's relatively new social hierarchy (Wooddell et al., 2017; 2019). The group remained there until it was disbanded in August 2016 (when all subjects were approximately 17-months old), again due to management decisions. Thus, the total duration of the study was eight months (Figure 1), with approximately 18 weeks of pre-SI and 10 weeks of SI.

Behavioral data collection: aggression, dominance ranks, and dominance certainty

Detailed methodologies for dominance data collection in this group have previously been reported (see Wooddell et al., 2017; 2019). In addition to direct aggressive interactions, coalitionary support was also recorded, indicating which individual they supported in the aggressive interaction (see Wooddell et al., 2017). Coalitionary support was totaled for each dyad, in counts across each study period for initiating coalitionary support to another partner and counts for receiving coalitionary support from another partner. Elo-rating, a method commonly used to track rank changes over time (Neumann et al., 2011) was used to establish dominance ranks, which has previously been documented in detail for this group (Wooddell et al., 2017; 2019).

Contrary to dominance rank, DC is a relatively new method that utilizes both direct and indirect information via social network analysis to gauge the overall "fit" of an individual's position in the dominance network (Fushing et al., 2011; Linden et al., 2019; Schrock et al., 2019; Vandeleest et al., 2016; 2019). DC was calculated from dominance interactions (threats, chases, attacks, displacements, silent-bared teeth displays) using the percolation and conductance method implemented via the Perc package (Fujii et al., 2015; Vandeleest et al., 2016) in R software (v 3.3.3) This is a network-based method that handles missing data better than other ranking methods by measuring both direct and indirect pathways and transitivity in the dominance networks. In other words, the Perc package computes dominance information from all network pathways, even for those pairs that were never recorded interacting, measuring the probability of each individual outranking another. Therefore, individuals high in DC indicate that individuals are more certain of their rank position compared to individuals with low DC. Values typically range from 0.5 (uncertain position; an individual has an equal probability of ranking higher or lower than another individual) to 1 (certain position; an individual has a 100% probability of ranking higher than another individual). Previous studies in have reported an inverted-U function of DC, where both low- and high-ranking individuals have the greatest DC (i.e., the most consistent or stable dominance position), with the most uncertainty (i.e., the greatest inconsistency or unstable dominance position) typically exhibited by mid-ranking individuals (e.g., Schrock et al., 2019; Vandeleest et al., 2016).

Behavioral data: affiliation and social networks

Detailed methodologies for affiliative social behavior collection in this group have previously been reported (see Wooddell et al., 2017; 2019). For the purposes of this study, we

only focused on social grooming and social play, as these are common affiliative behaviors in juvenile macaques (Kulik et al., 2015).

Two separate social networks were constructed: one for social grooming and one for social play, both after group formation and after relocation/SI (thus totaling four separate networks). To construct social networks, we totaled the number of intervals in which each subject engaged in either social grooming or social play with each social partner. We then used weighted and undirected information to create social networks using the "Statnet" and "SNA" (Handcock et al., 2006) packages in R software (R Core Team, 2020; v 3.3.3). We used the *evcent* function to calculate weighted eigenvector centrality (EC) for each individual, for both social grooming and social play. EC measures the strength of an individual's direct connections, but also the strength of its indirect connections (i.e., how socially integrated an individual's social partners are; Bonacich, 2007; Farine & Whitehead, 2015), and higher EC indicates that individuals are more socially connected within the group. EC has been found to be associated with several measures of fitness, such as female fertility (e.g., Brent et al., 2013), offspring survival (e.g., Cheney et al., 2016), biomarkers of inflammation (e.g., Wooddell et al., 2019), and survival (e.g., Stanton & Mann, 2012).

Hair cortisol concentrations (HCCs)

Owing to the longitudinal nature of this study, we utilized HCCs in the present study. Short-term samples such as blood and saliva are influenced by circadian rhythm and even the sampling procedure itself, whereas HCCs are not (Davenport et al., 2006; Meyer & Novak, 2012; Meyer et al., 2014). Similarly, cortisol concentrations in urine and feces only reflect HPA axis activity up to 24 hours, which would require numerous samplings over the course of the study, whereas HCCs are a cumulative measure over several weeks/months (Davenport et al., 2006;

Meyer & Novak, 2012; Meyer et al., 2014). Hair samples were collected longitudinally at routine quarterly health exams for all subjects from birth onwards following a shave, re-shave procedure. For the purposes of this study, the baseline shave occurred one day before group formation on January 18, 2016 (when subjects were approximately 8 months old). The re-shaves occurred three months after peer group formation on April 25, 2016 (when subjects were approximately 13 months old), and again on July 11, 2016 (when subjects were approximately 15 months old). These re-shave samples thus reflect the formation of the hierarchy and social networks (April 2016; see Figure 1), as well as SI that occurred after the relocation to a new housing environment on May 30, 2016 (the July 2016 samples; see Figure 1). For the purposes of this study, we report on April and July samples (or a change between those two samples). Samples were collected by shaving the back of the animals' necks with commercial pet grooming clippers and were stored in an aluminum pouch at -80°C until further processing. Samples were assayed according to previously established procedures (Davenport et al., 2006; Meyer & Novak, 2012; Meyer et al., 2014). In short, samples were washed twice with 5 mL isopropanol alcohol and dried under a fume hood for 5-7 days until being ground to a fine powder with a ball-mill grinder (MM200; Retsch, Newton, PA). Samples were then rotated in methanol for 24 hours and aliquots of the methanol extract were dried down and reconstituted with assay buffer, then analyzed with an enzyme immunoassay using a salivary cortisol kit (#1-3002; Salimetrics, Carlsbad, PA). Resulting values (µg/dL) were converted to pg/mg for analysis. Inter-assay and intra-assay coefficients of variation were <9.0%. One subject's hair sample for April 2016 was spilled during processing and is thus missing from analysis.

Statistical Analysis

We used the R function *glmmTMB* to run generalized linear mixed model (GLMM) analysis to test our predictions. However, before running the GLMM analysis, we first conducted an exploratory analysis to assess the presence of outliers and collinearity between predictors using the R package "HighstatLibV8.R" provided by Zuur et al. (2013). Outlier presence was assessed using the function *Mydotplot* while collinearity was tested using the functions *Mypairs* and *Mybwplot*. While this exploratory analysis did not reveal any outlier, we did find some predictors that were correlated: (1) social grooming and social play eigenvector values were negatively correlated ($r(16) = -0.63$, $p < 0.001$); (2) both social grooming and social play eigenvector values varied with early social experience condition, with MG infants having higher social grooming EC and lower social play EC than UG infants (social grooming: $t(16) = 5.2$, $p < 0.001$; social play: $t(16) = -3.35$, $p = 0.002$); and (3) DC varied with relocation, with mean DC values being significantly higher before relocation than after ($t(16) = -2.84$, $p = 0.008$). Following this exploratory analysis, we tested the effect of social grooming and social play on the outcome variable in separate models, and we excluded early social experience conditions in models that included either social grooming or social play as predictors. Similarly, we excluded relocation condition when DC was included as predictor.

To test prediction 1, we ran a GLMM analysis in which we set DC as the outcome variable with beta distribution (since DC values can only range between 0 and 1) while dominance rank (Elo-rating), relocation (before vs after), and early social experience (UG vs MG) were included as predictors (see Table 1). Similarly, we ran two separate GLMM models to address prediction 2a. While both models included DC values as outcome variables, the models had different predictors, with one model including, as an explanatory variable, social grooming EC and relocation, whereas a second model included social play EC and relocation (see Table 1).

Additionally, in order to assess whether EC was associated to the number of coalitionary supports individuals were involved in (prediction 2b), we ran six negative binomial GLMM models. These models included either the number of coalitionary support given, the number of coalitionary support received, or total number of coalitionary supports as outcome variables with a negative binomial error structure because the Poisson models were too overdispersed (Dispersion values > 2). We then set relocation and either social grooming EC or social play EC as predictors (see Table 1). To assess the effect of DC (prediction 3), as well as social network position (prediction 4) on HCCs, we ran linear mixed model analysis (LMM) with Gaussian distribution using the *lmer* function. In all three LMM models HCCs were included as an outcome variable, while each model had a different set of predictors: 1) DC and early social experience in one model; 2) relocation and social grooming EC in a second model; and 3) relocation and social play EC in a third model (see Table 1). All the mixed model analyses included subjects' IDs as random factors in order to control for pseudoreplication since the same individuals were sampled twice (both before and after relocation). For all the analyses, we ran each model three times: one as a null model (i.e., with only the random factors but with no predictors); one with predictors entered as main effects, and one with predictors entered as interaction. We then, for each analysis, compared Akaike's information criterion (AIC) values between the three models in order to find the model with the best fit (i.e., with the lowest AIC value). Furthermore, we used the *check_model* function from the "performance" package to verify that all (G)LMM models met the necessary assumptions of model validity (i.e., distribution of residuals, residuals plotted against fitted values). Finally, we used regression analysis to assess whether social grooming and social play EC during SI significantly predicted changes in HCCs after relocation compared to before relocation. To this end, we calculated, for

each individual, the percentage of changes in HCCs between the two time points (i.e., after group formation and after group relocation) by dividing the difference in HCCs between the two time points by the HCCs values after group formation and multiplying the ratio by 100. This percentage was included as the outcome variable in two regression models. In each model, we included either social grooming or social play EC during SI along with the early social experience (UG or MG) as a control variable. Each of these regression models were run twice, both with and without (i.e., null model) the main predictors. AIC values between the two models were compared in order to find the model with the best fit. In all models, we z-transformed continuous predictors in order to better compare effect sizes of variables that are on different scales.

Results

Prediction 1: Dominance certainty should decrease during social instability

Our GLMM analysis showed that the model that included the three-way interaction between Elo-rating, relocation (before vs after), and early social experience (MG vs UG) had a better fit compared to both the model in which the three predictors were set as main effects and the null model (Table 2 and S1). This analysis revealed that the three-way interaction significantly predicted DC (estimate= -1.60, SE=0.32, z-value=-4.97, $p<0.001$, Table 2). In particular, before relocation, for both MG and UG, higher-ranking subjects had greater DC than lower-ranking subjects, but this effect was much stronger for MG than for UG subjects (Figure 2a). In contrast, after relocation, the situation was reversed for MG, with higher-ranking MG subjects displaying less DC than lower-ranking MG subjects, while higher-ranking UG subjects had greater DC than lower-ranking UG subjects (Figure 2b). These results (partially) support prediction 1.

316 *Prediction 2: Individuals with greater centrality in social grooming and social play networks*
317 *should have greater dominance certainty*

318 Our GLMM analysis to test prediction 2 revealed that the models that included the
319 predictors as main effects had a better fit compared to the null model as well as the models with
320 the interaction term (Tables S2 & S3). This analysis showed that, while social grooming EC did
321 not predict variation in DC, there was a negative relationship between social play EC and DC,
322 (Tables 3 & 4), indicating that individuals who were more peripheral in the play network had
323 somewhat higher DC. Overall, these results fail to support prediction 2a.

324 In the GLMM models we ran to assess whether the position in the social grooming
325 network significantly predicted the number of coalitionary supports, we found that models with
326 the interaction between network position and relocation had a better fit compared to the null
327 model and the model with predictors set as main effects (Tables S4-S6 & Table 5). This analysis
328 shows a positive relationship between social grooming EC and coalitionary support before
329 relocation, but a negative relationship *after* relocation (i.e., after the onset of SI; Figures 3a-c). In
330 contrast, we found that social play EC significantly predicted only the number of coalitionary
331 supports received (vs. coalitionary support given and total coalitionary support; Tables S7-S9
332 and Table 6). This model revealed a negative relationship between social play EC and
333 coalitionary support received before relocation and a positive (albeit weak) relationship after
334 relocation (Figure 4). Collectively we found some support for prediction 2b, with coalitionary
335 support positively correlated with social grooming EC only before relocation and with social
336 play EC only after relocation.

337 *Prediction 3: Individuals with less dominance certainty should have higher hair cortisol*
338 *concentrations*

HCCs ranged from 49.84 pg/mg to 160 pg/mg in April 2016 before relocation, to between 58.58 pg/mg and 113.17 pg/mg after relocation in July 2016 (before: M= 92.82, SD = 23.80; after: M = 85.92, SD = 15.07). The change in HCCs ranged from -81.01 to 34.22 pg/mg (M= -8.07, SD = 28.13)

GLMM analysis revealed that the model that included the interaction between DC and early social experience (MG/UG) had a better fit compared to the null model and the model in which predictors were set as main effects (Table S10). However, we did not find any significant effect of either DC or early social experience on HCC values (Table 7). Overall, we found no support for prediction 3.

Prediction 4: Individuals more central in social grooming and social play networks should have lower HCCs

We have previously reported that rates of social grooming significantly increased following relocation and SI, whereas rates of social play significantly decreased (Wooddell et al., 2017). We also previously reported that there was no significant change in social grooming EC values during SI (Wooddell et al., 2019; i.e., individuals did not change in their social connectedness during SI). Rather, social grooming EC values before and after SI were highly correlated ($r(16) = 0.87$, $p<0.0001$), similar to social play EC values ($r(16) = 0.63$, $p = 0.005$), indicating that individuals had similar social network positions before and during SI.

Data from the current study partially supported prediction 4. The GLMM models that included the interaction between affiliative EC (social grooming and social play) and relocation (before/after) had the best fit (Tables S11 & S12), but neither social grooming nor social play EC values individually (nor their interactions with relocation) significantly predicted HCCs (Tables

8 & 9), failing to support prediction 4. However, after the relocation, young monkeys' EC in the social play network negatively predicted the percentage of change in HCCs from April 2016 to July 2016. This relationship approached significance and explained ~ 20% of the variation in the difference in HCC values between before and after relocation (estimate= -14.0, SE=6.8, t(16)=-2.05, p=0.060, R^2= 0.22; Figure 5). In other words, juveniles that were more central in the social play network (i.e., had more direct and/or indirect connections) exhibited smaller increases in HCCs levels during SI. Monkey's EC in the social grooming network did not predict changes in HCCs (Table S13). Collectively, these data partially support prediction 4.

Discussion

In our study of behavioral and hormonal changes during SI in young macaques, we found that DC decreased during a period of social instability. Individuals that were socially peripheral in the social play network had increased DC. Before SI, individuals more central in social grooming networks received more frequent coalitionary support, although this relationship was opposite during SI. Individuals more central in social play networks received less frequent coalitionary support prior to SI, but more frequent coalitionary support during SI. DC did not predict HCCs, rather affiliation in social networks seemed to buffer individuals during SI. Yearlings who were more central in their social play network appeared to exhibit smaller increases in chronic cortisol production during a period of SI. We interpret this finding to mean that juveniles with more direct and/or indirect connections (i.e., more "friends") may be buffered against the physiological stress of social instability. We now discuss the relevance and possible explanations of these findings.

As predicted, DC decreased following a period of SI. Therefore, as the subordinate (UG) peers challenged the dominant (MG) peers, there was generally an overall increase in the

384 presence of ambiguous relationships. This is particularly evident, as prior to SI, higher-ranking
385 MG subjects had greater DC than lower-ranking MG subjects. However, during SI, higher-
386 ranking MG subjects and lower-ranking UG had lower DC than lower-ranking MG subjects and
387 higher-ranking UG subjects, respectively. This is likely because higher-ranking MG subjects and
388 lower-ranking UG subjects were now the middle-ranking animals, and ranks were unsettled
389 among the highest-ranking MG subjects and the lowest-ranking UG subjects, as they were
390 challenging each other, whereas ranks among the MG subjects remained perfectly stable
391 (Wooddell et al., 2017). This is consistent with other literature indicating mid-ranking animals
392 typically have the lowest DC (Schrock et al., 2019; Vandeleest et al., 2016).

393 Surprisingly, individuals more peripheral in social play networks had increased DC, as
394 these individuals may have been high- or low-ranking to begin with, which often have certain
395 dominance positions (Schrock et al., 2019; Vandeleest et al., 2016). In addition, given that play
396 fighting can quickly escalate to real fighting (Paquette, 1994), social play may have been too
397 risky, as dominance ranks were being established. Therefore, individuals less connected in social
398 play networks may have been more certain of their dominance ranks, because they did not
399 engage in risky play behavior, as social play can also be used as a way to assess competitors
400 (Cordoni et al., 2021), which would be useful information to have in case of a rank challenge. In
401 addition, we had predicted that individuals with more social connections might have benefitted
402 from a larger network of coalitionary support, making their rank position more certain (i.e.,
403 higher DC). Prior to SI, yearlings more central in social grooming networks received more
404 frequent coalitionary support. Similarly, a broad range of studies has shown that grooming is
405 traded for agonistic support in several primate species (Carne et al., 2011; Hemelrijk, 1994,
406 Kaburu and Newton-Fisher, 2015; Schino et al., 2007; Seyfarth and Cheney, 1984; Silk, 1992),

but our work provides preliminary evidence that such exchange might emerge early in life, which is an exciting avenue for continued research. During SI, however, individuals more central in social grooming networks received less frequent coalitionary support. There are several possible explanations, all which require follow-up study. First, during SI, it is possible that social relationships and "trust" may have been broken down, and they must be re-established, which may have been done through grooming (e.g., Kaburu & Newton-Fisher, 2013), which is a common way to solidify social bonds (McFarland, 2018). Similarly, it is possible that during SI, individuals focused more on social grooming as a way to indicate social support, rather than providing energetic and costly coalitionary support. Given that social grooming can lower heart rates and reduce tension, the increased rates of social grooming during SI (Wooddell et al., 2017) may have been a needed mechanism to help reduce tension and reduce distress during SI (Aureli & Yates, 2010). Finally, it is possible that individuals connected in the social grooming network may not have needed coalitionary support, as they were not as engaged in the fighting.

We did, however, find that coalitionary support was negatively related to social play EC before and during SI, which is the opposite of the finding on social grooming. This may be because the two social networks were negatively correlated with each other. Given the dissociation between the two networks, this might indicate that although both of these behaviors are considered "pro-social", they may have different underlying functions, similar to how social grooming can have several different functions depending on context (e.g., parasite reduction, thermoregulatory, tolerance/exchange for other commodities, maintain social cohesion: McFarland, 2018), whereas social play can refine motor skills (Palagi, 2018), maintain social relationships (Shimada & Sueur, 2018), and assess the strength of opponents (Cordoni et al., 2021). While play bouts are often short and require an equal investment between the individuals

involved, grooming often requires a substantial investment in time and effort and is mainly disproportionately performed by only one member of the pair. Animals may make economic decisions by exchanging cooperative behaviors on the basis of how much they are willing to invest in a relationship (Noe et al., 2001), and grooming might offer a more honest signal than play, which might explain why grooming, rather than play, was exchanged for coalitionary support during the group formation, which was reversed during SI. Furthermore, if social play can be a way to "test competitors," (Cordoni et al., 2021) more socially connected individuals in the social play network could be receiving more frequent coalitionary support, as individuals are providing support to strong competitors. By forming alliances with strong competitors, this could result in a cooperative exchange of coalitionary support.

Contrary to our predictions, we did not find that lower DC predicted higher HCCs, as there were no significant main effects or interactions with DC on HCCs. First, there was high variability in HCCs across the study period, which is partially due to the small sample size. This high variability thus indicates that other factors, still unknown to us, predicted HCCs. Second, although we found little overt behavioral evidence of SI following the formation of the social group, as the hierarchy was formed in less than 30-minutes (Wooddell et al., 2017), the formation of a novel social group is most certainly a stressor (see Dettmer et al., 2012). For all the subjects in this study, this group formation was compounded by the effects of the overthrow (MG subjects) and/or removal of caregivers (all subjects). Thus, the group formation itself may have contributed to the variable HCCs in the period before SI. Given that cortisol is a metabolic hormone that regulates energy, HCCs may be elevated in general due to higher activity budgets in a group formation process.

SI did result in changes in social behavior. Similar to adults, rates of social grooming in the peer group significantly increased (i.e., the frequency tripled) during SI, potentially as a coping mechanism (Engh et al., 2005; Schino et al., 1988; Wooddell et al., 2016), whereas rates of social play significantly decreased (e.g., the frequency nearly halved), possibly due in part to the risky nature of social play interactions (e.g., Paquette, 1994), which may be further escalated during a new group formation. However, individuals' social network positions (measured via EC) were rather stable across the study. This finding indicates that juveniles did not expand their social network during SI. Therefore, it is likely that peers did not change their social connections (for an example, see Testard et al., 2021), but instead strengthened (via enhanced rates of grooming) their connections with their preferred social partners, which requires follow-up study. This may have been advantageous, as previous research has indicated that strong social bonds with a few social partners have consequences for both GC production (Wittig et al., 2008) and longevity (Silk et al., 2009; 2010).

A novel finding of this study is the potential buffering effect of social play during SI. Although neither social grooming nor social play eigenvector values predicted HCCs at either time point separately (although they were the best fit models), the social play values during SI predicted a *smaller increase* in HCCs during SI. This finding indicates that although social play was potentially costly and occurred at a lower frequency during SI, social play was still important and individuals that were well connected and could "afford" to engage in social play may have realized some benefits. In addition to the social benefits of receiving more frequent coalitionary support during SI, the benefits of social play may have also been physiological. For example, social play is highly rewarding, modulated by the nucleus accumbens and dopaminergic transmission (Manduca et al., 2016), which can alter dendritic morphology and

susceptibility to social stress (Burleson et al., 2016). Our findings are consistent with previous work showing that social play is associated with decreased cortisol and stress behaviors (Biben & Champoux, 1999; Norscia & Palagi, 2011; Wooddell et al., 2017), even when individuals were experimentally exposed to social stressors (e.g., exposure to an unfamiliar human subject: Schöberl et al., 2016), or social separations (e.g., Mustoe et al., 2014). Thus, there may be a negative feedback loop, whereby stress limits but does not extinguish social play, and social play also limits stress. For young mammals, in which social play is a predominant behavior, social play can be particularly important in the regulation of the HPA axis. Thus social instability itself may not be particularly stressful for young primates, but rather how they *behaviorally adapt* may be the more important factor. Given that juvenile macaques engage in social play frequently (Kulik et al., 2015) which decreases as they transition into adulthood, social play may be an important regulator of the HPA axis in juvenility, which may then switch to social grooming in adulthood (e.g., of social grooming on HCCs: Wooddell et al., 2016; 2017). Of course, future research should corroborate our preliminary findings.

Our study is not without its limitations. The most obvious limitation is this study's sample size. Studies that focus on SI are often opportunistic and are thus retrospective, limiting our ability to have large sample sizes and multiple variables to test and control for. Replication is therefore needed in larger cohorts and colonies where other variables (e.g., sex) can be explored and experimentally controlled. Another limitation is this study was not conducted in a naturalistic group in which there are multiple sources of kin (and nonkin). Given that kin (especially mothers) can influence offspring social networks (e.g., Timme, 1995; Wooddell et al., 2019), young primates can respond to SI either by direct influences of the mother and other kin or indirectly by mimicking the actions of close family. Also, mothers can act as social buffers by

498 providing a meaningful form of social support that can mitigate the stress response (Sanchez et
499 al., 2015). SI in a naturalistic group also contains multiple individuals and families fighting each
500 other, which would likely be much more salient than group formation for peer group of ~1 year-
501 olds, which could explain the lack of effects of SI and DC on HCCs. Therefore, future research
502 should seek to examine social SI in a naturalistic group.

503 However, our study is advantageous in beginning to examine how juveniles respond to SI
504 in the absence of the influence of other adult kin. These findings provide a preliminary analysis
505 for future studies about how personality, genetics, and early social experiences can lead to
506 coping strategies, all of which can provide important translational research for humans. Another
507 limitation is that, given the opportunistic nature of this study, our sex ratio was heavily skewed
508 towards males (14 males, 4 females), thus not allowing us to draw conclusions about potential
509 sex differences in behavioral and hormonal adaptation to SI. Given that juvenile females direct
510 their social interactions to adult kin, particularly grooming, whereas males are more involved in
511 play interactions, particularly with peers (Kulik et al., 2015), the sexes may employ different
512 behavioral strategies in handling SI, although we were unable to study sex differences due to this
513 skewed sex ratio. Future research should include sex as an effect when examining how juveniles
514 respond to SI. Moreover, a larger observation time would have been ideal, but unexpected
515 managerial decisions precluded this. Finally, given that we studied a yearling group immediately
516 after group formation, although the behavioral data indicated the group was stable (especially
517 compared to after relocation), an ideal comparison would have been studying a long-term social
518 group during stability and instability. Given that group formations are often used as captive
519 studies of SI, this brings into question whether all types of group formations really are forms of
520 SI. Future work will be able to address these questions.

Conclusion

SI may occur rather frequently in the social lives of adult primates (particularly males), although considerably less is known about how SI influences behavioral and hormonal development in juveniles. We found that yearling rhesus macaques respond to SI with changes in social behavior, and that social play may have acted as a potential social buffer in regulating the HPA-axis. This research adds to the growing body of literature of the importance of social play in the lives of developing primates and highlights the need for continued research.

Acknowledgements

The data presented in this study were collected at the Laboratory of Comparative Ethology, which was funded by the Division of Intramural Research at the Eunice Kennedy Shriver National Institute of Child Health and Human Development. The funding agency had no role in study design, collection, analysis, interpretation, writing, or intent to submit this article. The authors have no conflicts of interests to declare. We thank Dr. Stephen J. Suomi for his support of the project. We also thank Ashley M. Murphy for assistance with data collection and Kristen Byers, Ryan M. McNeill, and Emily Slonecker for assistance with hair sampling. We are grateful to Jerrold S. Meyer's lab at University of Massachusetts Amherst for assaying the July 2016 hair samples. We also thank the animal care and veterinary staff at the NIH Animal Center, with whom this research would not have been possible.

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808

Table1

Table 1. Summary of the models used to test our predictions. All mixed model analyses included subject ID as random factor in their model.

Type of analysis	Prediction	Outcome variable	Predictors
GLMM (Beta distribution)	1. Effect of SI on DC	DC	Elo-rating Relocation Early social experience
GLMM (Beta distribution)	2a.Effect of grooming EC on DC	DC	Grooming EC Relocation
GLMM (Beta distribution)	2a.Effect of play EC on DC	DC	Play EC Relocation
GLMM (Negative binomial distribution)	2b.Effect of grooming EC on support given	Number of support given	Grooming EC Relocation
GLMM (Negative binomial distribution)	2b.Effect of grooming EC on support received	Number of support received	Grooming EC Relocation
GLMM (Negative binomial distribution)	2b.Effect of grooming EC on total support	Number of total support (given + received)	Grooming EC Relocation
(GLMM (Negative binomial distribution)	2b.Effect of play EC on support given	Number of support given	Play EC Relocation
GLMM (Negative binomial distribution)	2b.Effect of play EC on support received	Number of support received	Play EC Relocation
GLMM (Negative binomial distribution)	2b.Effect of play EC on total support	Number of total support (given + received)	Play EC Relocation
LMM (Gaussian distribution)	3.Effect of DC on HCC	HCC	DC Early social experience
LMM (Gaussian distribution)	4.Effect of grooming EC on HCC	HCC	Grooming EC Early social experience
LMM (Gaussian distribution)	4.Effect of grooming EC on HCC	HCC	Play EC Early social experience

GLMM = Generalized Linear Mixed Model; LMM = Linear Mixed Model; SI = Social Instability; DC = Dominance Certainty; EC = Eigenvector Centrality

Table 2. Results of the GLMM analysis (n=18) examining whether the three-way interaction between Elo-rating, relocation (before vs after) and early social experience (UG vs MG) significantly predicted DC. Significant predictors are indicated in bold.

Predictors	Estimate	SE	z-value	p-value
Intercept	2.00	0.13	15.69	< 0.001
Elo-rating (z-transformed)	**-0.57**	**0.14**	**-3.97**	**< 0.001**
Relocation	0.16	0.19	0.86	0.390
Early experience	**-0.40**	**0.17**	**-2.40**	**0.016**
Elo-rating * Relocation	**1.43**	**0.18**	**7.79**	**< 0.001**
Elo-rating * Early social experience	**1.07**	**0.20**	**5.37**	**< 0.001**
Relocation * Early social experience	**0.93**	**0.27**	**3.39**	**< 0.001**
Elo-rating * Relocation * Early social experience	**-1.60**	**0.32**	**-4.97**	**< 0.001**

Table 3. Results of the GLMM analysis (n=18) examining whether grooming eigenvector centrality (EC) and relocation (before vs after) significantly predicted dominance certainty. Significant predictors are indicated in bold.

Predictors	Estimate	SE	z-value	p-value
Intercept	2.04	0.12	16.62	< 0.001
Grooming EC (z-transformed)	0.13	0.11	1.22	0.224
Relocation	**0.47**	**0.18**	**2.56**	**0.011**

Table 4. Results of the GLMM analysis (n=18) examining whether play eigenvector centrality (EC), relocation (before vs after) and early social experience (UG vs MG) significantly predicted dominance certainty. The significant predictor is indicated in bold.

Predictors	Estimate	SE	z-value	p-value
Intercept	2.02	0.12	17.20	< 0.001
Play EC (z-transformed)	**-0.18**	**0.09**	**-2.13**	**0.033**
Relocation	**0.53**	**0.18**	**2.96**	**0.003**

Table 5

Table 5. Results of the GLMM analysis (n=18) examining whether grooming eigenvector centrality (EC), and relocation (before vs after) significantly predicted the number of coalitionary supports given, received and total. Significant predictors are indicated in bold.

	Predictors	Estimate	SE	z-value	p-value
Support given	Intercept	3.10	0.17	18.51	< 0.001
	Grooming EC (z-transformed)	-0.22	0.17	-1.30	0.195
	Relocation	**-0.95**	**0.25**	**-3.86**	**< 0.001**
	Grooming EC * Relocation	**0.54**	**0.25**	**2.17**	**0.030**
Support Received	Intercept	2.32	0.13	18.43	< 0.001
	Grooming EC (z-transformed)	0.22	0.13	1.76	0.079
	Relocation	**0.75**	**0.13**	**5.70**	**< 0.001**
	Grooming EC * Relocation	**-0.41**	**0.14**	**-3.01**	**0.003**
Total support	Intercept	2.93	0.14	21.37	< 0.001
	Grooming EC (z-transformed)	0.28	0.14	1.92	0.055
	Relocation	**0.83**	**0.16**	**5.23**	**< 0.001**
	Grooming EC * Relocation	**-0.49**	**0.16**	**-2.99**	**0.003**

Table 6. Results of the GLMM analysis (n=18) examining whether play eigenvector centrality (EC) and relocation (before vs after) significantly predicted the number of coalitionary supports given, received and total. Significant predictors are indicated in bold.

	Predictors	Estimate	SE	z-value	p-value
Support given	Intercept	2.21	0.19	11.65	< 0.001
	Play EC (z-transformed)	-0.04	0.13	-0.32	0.749
	Relocation	**0.92**	**0.26**	**3.50**	**< 0.001**
Support received	Intercept	2.34	0.13	17.54	< 0.001
	Play EC (z-transformed)	-0.15	0.16	-0.96	0.338
	Relocation	**0.77**	**0.14**	**5.51**	**< 0.001**
	Play EC * Relocation	**0.33**	**0.17**	**1.97**	**0.049**
Total support	Intercept	2.99	0.14	21.27	< 0.001
	Play EC (z-transformed)	-0.02	0.10	-0.24	0.808
	Relocation	**0.84**	**0.19**	**4.32**	**< 0.001**

Table 7. Results of the GLMM analysis (n=18) examining whether the dominance certainty (CC) and early social experience (UG vs MG) significantly predicted hair cortisol concentrations. Significant predictors are indicated in bold.

Predictors	Estimate	SE	z-value	p-value
Intercept	87.79	5.07	17.32	< 0.001
DC (z-transformed)	6.12	5.61	1.09	0.283
Early social experience	1.39	7.22	0.19	0.849
DC * Early social experience	-5.33	7.32	-0.73	0.472

Table 8. Results of the GLMM analysis (n=18) examining whether grooming eigenvector centrality (EC) and relocation (before vs after) significantly predicted hair cortisol concentrations.

Predictors	Estimate	SE	z-value	p-value
Intercept	92.79	4.74	19.60	< 0.001
Grooming EC (z-transformed)	0.66	4.62	0.14	0.887
Relocation	-6.76	6.58	-1.03	0.320
Grooming EC * Relocation	7.58	6.62	1.15	0.268

Table 9. Results of the GLMM analysis (n=18) examining whether play eigenvector centrality (EC) and relocation (before vs after) significantly predicted hair cortisol concentrations.

Predictors	Estimate	SE	z-value	p-value
Intercept	92.93	4.91	18.93	<2e-16
Play EC (z-transformed)	0.56	6.19	0.09	0.928
Relocation	-7.31	6.57	-1.11	0.285
Play EC * Relocation	-4.32	7.32	-0.59	0.561

Figure 1

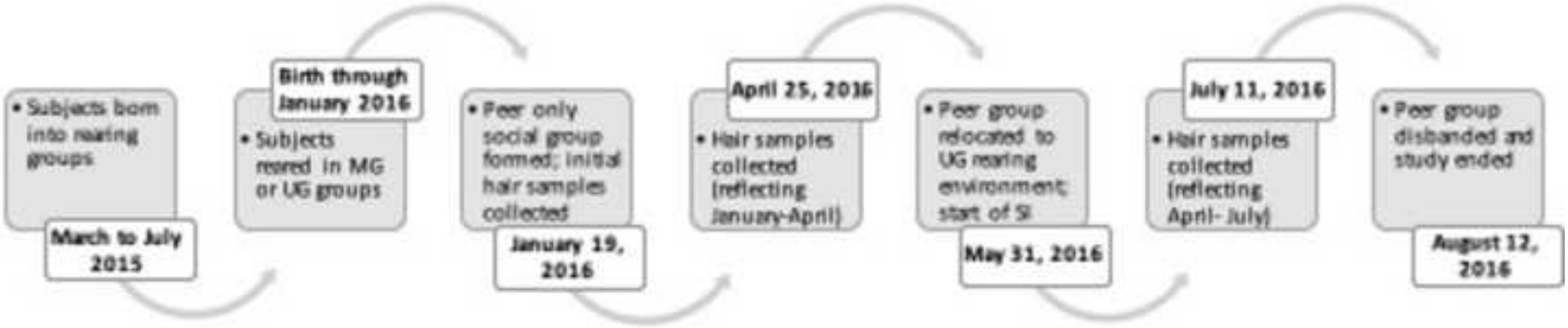
• Subjects born into rearing groups
March to July 2015
Birth through January 2016
• Subjects reared in MG or UG groups
• Peer only social group formed; initial hair samples collected
January 19, 2016
April 25, 2016
• Hair samples collected (reflecting January-April)
• Peer group relocated to UG rearing environment; start of SI
May 31, 2016
July 11, 2016
• Hair samples collected (reflecting April- July)
• Peer group disbanded and study ended
August 12, 2016

Figure 2

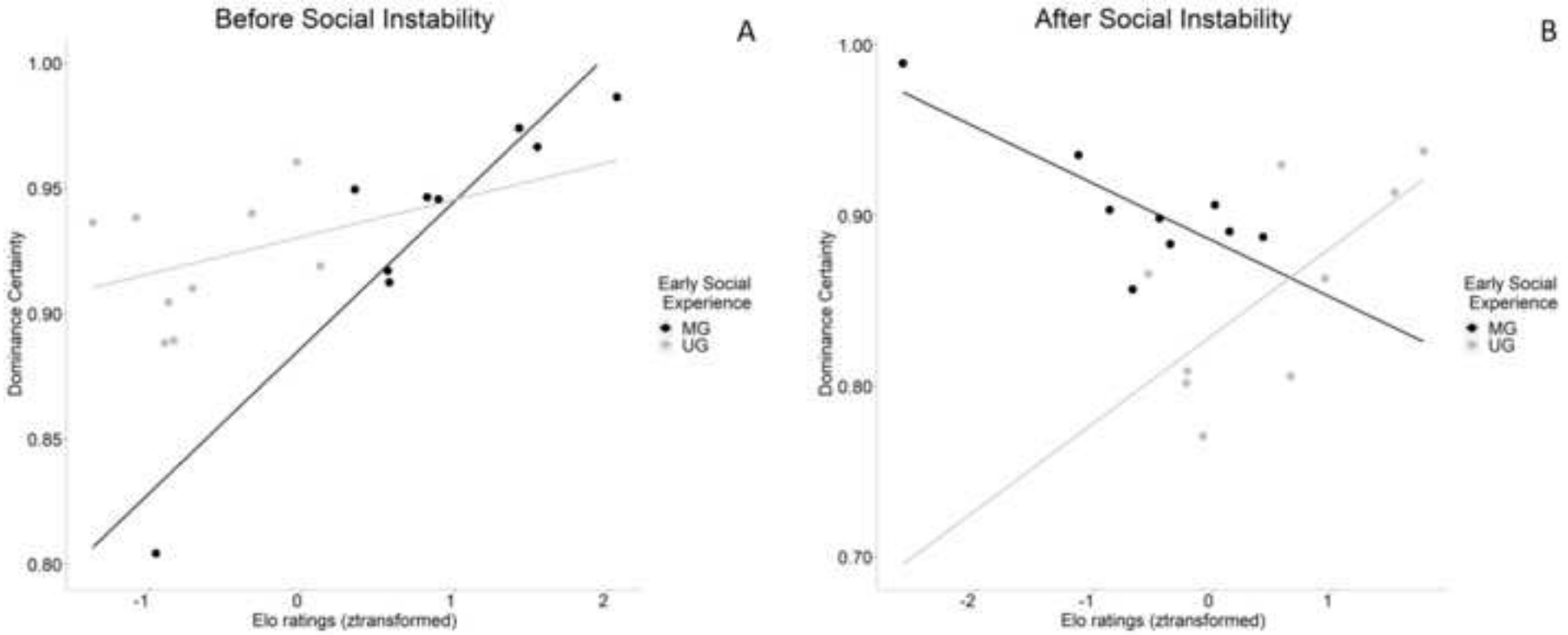
Before Social Instability
A
Dominance Certainty
1.00
0.95
0.90
0.85
0.80
-1
0
1
2
Elo ratings (ztransformed)
Early Social Experience
MG
UG
After Social Instability
B
Dominance Certainty
1.00
0.90
0.80
0.70
-2
-1
0
1
Elo ratings (ztransformed)
Early Social Experience
MG
UG

Figure 3

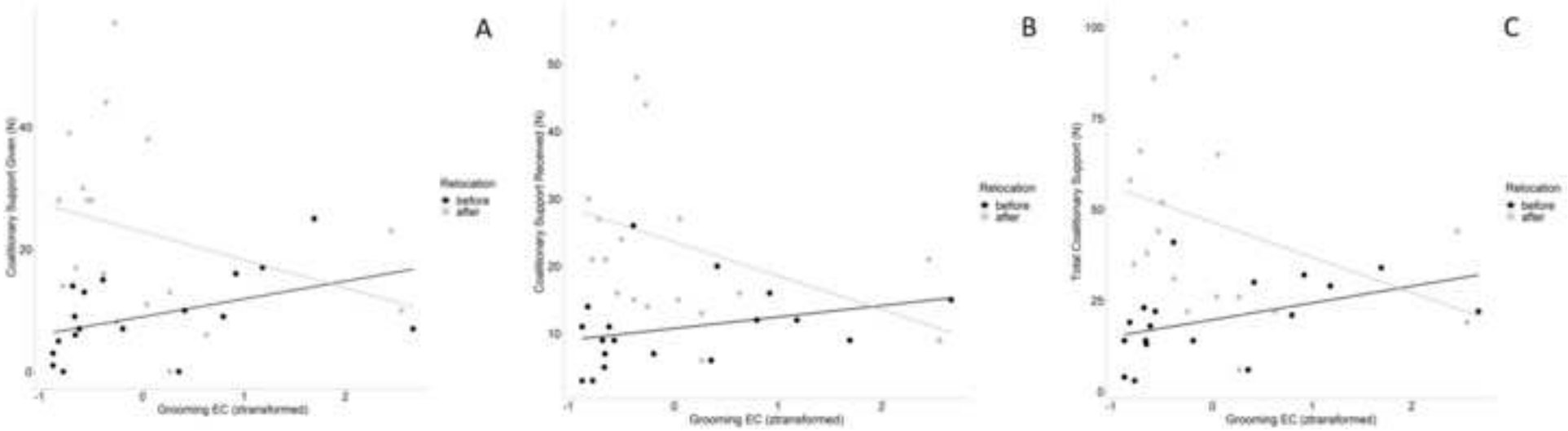
A
B
C
Coalitionary Support Given (N)
Coalitionary Support Received (N)
Total Coalitionary Support (N)
Grooming EC (ztransformed)
Relocation
before
after

Figure 4

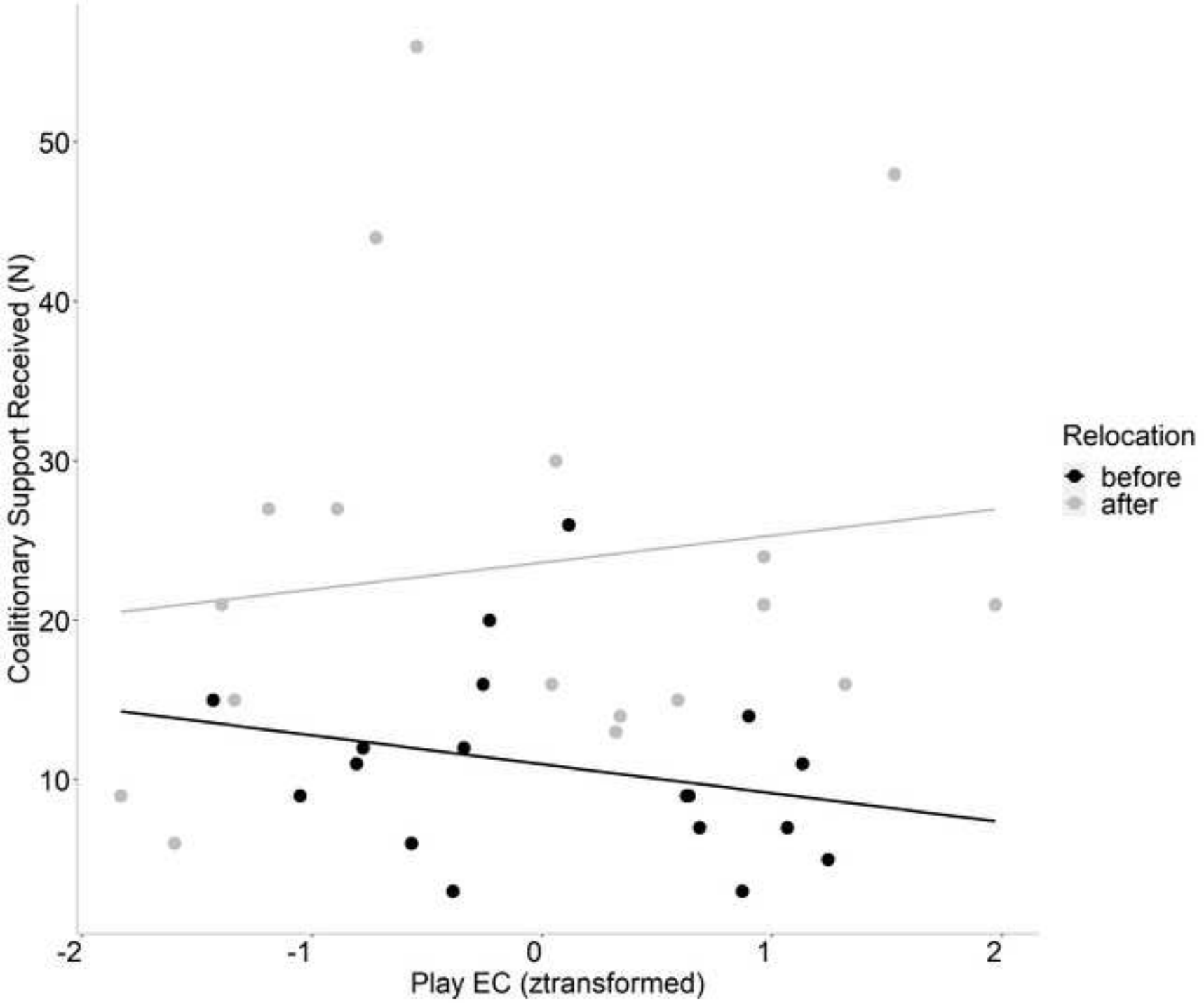
Coalitionary Support Received (N)
50
40
30
20
10
-2
-1
0
1
2
Play EC (ztransformed)
Relocation
before
after

Figure 5